Acidogenic Fermentation Towards Valorisation of Organic Waste Streams into Volatile Fatty Acids

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Anaerobic acidification of eight organic streams (cheese whey, sugarcane molasses, organic fraction of municipal solid wastes (OFMSW), glycerol, soapy slurry, winery wastewater, olive mill effluent, and landfill leachate) was evaluated in batch experiments to determine their acidogenic potential and examine the composition of the produced volatile fatty acids (VFA). Cheese whey, molasses and OFMSW presented the highest acidogenic potentials (0.3 to 0.4 gVFA per g of chemical oxygen demand fed, COD_{fed}) with the predominance of acetic, n-butyric and propionic acids. A further experimental set was applied to cheese whey, by varying food-to-microorganism ratio (F/M) and initial alkalinity. Maximisation of VFA production (up to 0.63 gVFA $g^{-1}COD_{fed}$) was obtained for an initial alkalinity of 5 – 7 g L^{-1} as $CaCO_3$ and F/M ratios of 2 – 4 gCOD g^{-1}VSS. Moreover, it was demonstrated that low F/M ratios combined with high alkalinity supply can shift the VFA profile by increasing the production of propionic and n-valeric acids. The results are useful towards optimal designs for acidogenic processes based on the composition of the VFA produced, since the control of the acidification products is crucial for valorisation in some applications.

Key words:
Anaerobic acidogenesis, volatile fatty acids (VFA), cheese whey, sugarcane molasses, OFMSW

Introduction

Anaerobic digestion (AD) has been applied over the years for the treatment of a wide variety of organic streams. The advantages of this process over other biological treatments relate mainly to the low cost of bioreactors operation, when compared to aerobic processes, since AD-based treatments offer a cost-effective solution for the treatment of high-strength organic wastes, through economic return of bioenergy (methane) and by-products generation.

AD is a sequential biochemical process in which the complex organic components present in the carbon waste, such as polysaccharides, proteins, and lipids, are hydrolysed, broken down, and fermented into intermediate products that are subsequently reduced into methane and carbon dioxide. These stages are commonly referred to as hydrolysis, acidogenesis and methanogenesis. In conventional AD applications, these transformations take place together in a single reactor system, where a delicate balance between the several groups of microorganisms is kept, although they differ widely in terms of physiology, nutritional needs, environmental sensitivity, and growth and substrate uptake kinetics.[1]

Problems in the stability and control of conventional AD systems have led to the technological development of new solutions, such as phase separation, firstly proposed by Poland and Gosh.[2] Two-phase anaerobic digestion implies a system configuration with separate reactors for acidogenesis and methanogenesis connected in series, thus allowing for the optimisation of each process separately. The first phase (acidogenic fermentation) leads to the production of intermediate products mostly volatile fatty acids (VFA), whereas the second (methanogenic phase) results in the conversion of these intermediates into stable end products, mainly methane and carbon dioxide. Although both anaerobic processes with and without phase separation are well developed, there is little information on adequate design and operation of acidogenic reactors.[3] Therefore microbiological and kinetic aspects on the operation of acidogenic reactors with

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different complex substrates should be better evaluated for an industrial exploitation of this process.[1]

In an open microbial mixed culture operating with complex substrates, many types of microorganisms and biochemical pathways are involved in the acidogenesis process, so a large number of by-products can be formed, including carboxylic acids. This fact makes the acidogenic fermentation of organic feedstocks (industrial and agricultural waste streams) an interesting way to generate valuable intermediate chemicals. Although methane is usually considered the final product of an anaerobic treatment, other valuable by-products can compete with it, such as the volatile fatty acids (VFA). Currently, these carboxylic acids constitute a valuable resource for biodegradable polymer production (polyhydroxyalkanoates, PHA),[4] biological nutrient removal,[5] or building blocks used as starting materials for the chemical industry.[6] Additional innovative applications for VFA include their thermal conversion to ketones and subsequent hydrogenation to alcohol fuels,[7] or even their use as electron donors in microbial fuel cells.[8] As most other commodity chemicals, VFA are currently being produced from fossil fuels through chemical synthesis. However, acidogenic fermentation, using renewable carbon resources, is a preferable strategy from the viewpoint of sustainable development. Therefore, there is an opportunity to shift AD by-products from simple intermediates to valuable end-products, thus proposing the concept of carboxylate platform.[9]

Even though acidification processes are virtually applicable to a wide variety of organic streams, few developments have been made on the perspective of VFA recovery, whereas much more attention has been paid to methanogenic microorganisms due to their higher sensitivity to inhibition and slower growth rates when compared to acidogenic microorganisms.[1,10] However, for the production of cost-effective amounts of VFA, it is necessary to design and configure bioprocesses adapted to circumvent the well-known VFA inhibition.[11] Additionally, the biochemical acidogenic potential of an organic waste stream, i.e. the amount of VFA that can be generated from fermentation of the organic constituents, as well as the knowledge of their profiles, are crucial parameters for the establishment of local-based biorefinery concepts able to produce value-added VFA.[12] Since the high kinetic rates for acidogenic metabolism can be an interesting feature for industrial exploitation of this process, cost-effective production of VFA will create new markets by providing new opportunities for the chemical industry.[6] Therefore, acidogenic fermentation constitutes a fast-moving field.

For some of the applications requiring VFA, the composition of fermentation products is a critical aspect when proposing the valorisation of organic waste streams into VFA. According to Serafim *et al.*[13], supplying different compositions as substrate for microbial production of PHA would result in polymers with different monomer composition, which is of major concern for commercial exploitation. Lemos *et al.*[4] demonstrated that a feeding stream rich in VFA with even carboxylic chains, such as acetic or butyric acid most of the time resulted in the formation of poly-3-hydroxybutyrate (PHB). In turn, the predominance of VFA with odd carbon atoms composition, such as propionate or valerate led to a copolymer with a higher 3-hydroxyvalerate (3HV) content, which is more interesting from a commercial viewpoint, regarding its mechanical properties.

This paper provides an assessment of the acidogenic potential of several organic waste streams commonly found in industrial processes (cheese whey, soapy residue, glycerol, sugarcane molasses, winery effluent, olive mill effluent, organic fraction of municipal solid wastes and landfill leachate), highlighting those which are more interesting for industrial exploitation, regarding the yields and the composition of the fermented streams. Cheese whey is a liquid by-product from dairy processing industry mostly consisting of lactose, lipids and proteins. Soapy slurry residue and glycerol are by-products from biodiesel synthesis with alkaline catalysts, where the glicerides are hydrolysed into glycerol and fatty acid salts characterised by surface-active properties (soapy emulsification). Sugarcane molasses is a by-product from sugar refinery and consists mostly of carbohydrates. Winery effluent consists mostly of polyphenols, sugars, tannins and vinification residues. Olive mill effluent contains polyphenols, carbohydrates and long-chain fatty acids. Leachate from municipal landfills consists of biodegradable and refractory organic matter, humic type constituents, ammonia, heavy metals and chlorinated salts. Organic fraction of municipal solid waste (OFMSW) consists of a mixture of residues with relatively high biodegradability characteristics due to the presence of food wastes.

The selection methodology aimed to choose streams which are widely available and currently represent threats to the conventional bio-treatment due to their high organic strength or inhibitory properties. In addition, a factorial experiment varying two operational conditions (F/M, food-to-microorganism ratio and initial alkalinity) in the acidogenic fermentation of one of the substrates that presented the highest acidogenic potential is presented and discussed, highlighting the combined effects on VFA production and profile.

Materials and methods

Organic waste streams

Two organic wastes (organic fraction of municipal solid waste, OFMSW; and soapy slurry resulting from biodiesel production), three organic effluents (olive mill effluent; winery effluent; and landfill leachate), and three organic by-products (sugarcane molasses; cheese whey; and wasted glycerol from biodiesel production) were selected for the evaluation of acidogenic potential through anaerobic batch experiments using anaerobic sludge as inoculum.

Cheese whey was collected from a medium scale factory producing cheese. Biodiesel by-products (soapy slurry residue and glycerol) were obtained from bench-scale experiments for biodiesel production by transesterification of vegetable oils. Sugarcane molasses was collected from a sugar refinery. Winery effluent was collected from a medium scale winery producing red wines. Olive mill effluent was collected from a small scale thermo-mechanical olive mill producing olive oil. Leachate was collected from a large scale multi-municipal landfill with an intermediate age. OFMSW was simulated as a typical mixture based on a year of residues collection in a typical Portuguese restaurant, with a composition based on Flor.[14] All the organic streams were stored at 4 °C before use. The main characteristics of all organic waste streams are presented in Table 1.

Inoculum

The inoculum consisted of a microbial mixed culture of anaerobic biomass collected from a full-scale mesophilic reactor (6000 m^3) that had been fed with wasted biological sludge from a wastewater treatment plant for domestic sewage and industrial effluents (50 % each in volume). The biomass was stored under anaerobic conditions at 4 °C until the beginning of the experiments, then it was washed, centrifuged and characterised prior to inoculation of each reactor.

Table 1 – *Organic content of the waste streams (mean ± standard deviation)*

	tCOD (g L^{-1})	sCOD (g L^{-1})	tCOD (g kg^{-1})	pH
Organic wastes				
OFMSW	–	–	380 ± 22	7.72 ± 0.82
Soapy slurry waste	211 ± 13.2	156 ± 18.7	–	9.65 ± 0.12
Organic effluents				
Olive mill effluent	55.70 ± 1.10	–	–	5.26 ± 0.34
Winery effluent	26.92 ± 2.15	–	–	3.45 ± 0.18
Landfill leachate	10.74 ± 0.85	10.16 ± 0.74	–	6.21 ± 0.12
Organic by-products				
Cheese whey	103 ± 13	98 ± 10	–	6.35 ± 0.11
Sugarcane molasses	952 ± 130	896 ± 102	–	5.83 ± 0.07
Wasted glycerol	–	–	2030 ± 73	9.21 ± 0.12

Experimental apparatus and conditions

Experiments were performed in batch mode, conducted in triplicate, using anaerobic glass reactors with a nominal volume of 320 mL, and a working liquid volume of 230 mL. To each reactor, 31 mL of anaerobic sludge were added to set a biomass concentration of 2 g L^{-1} of volatile suspended solids (VSS). The volumes or weights of the studied organic wastes were determined in order to achieve an initial substrate concentration corresponding to a COD of 8 g L^{-1}. Inorganic nutrients solution (according to van Lier *et al.*[15]), $NaHCO_3$ and $KHCO_3$ were added to provide mineral media and alkalinity of 2 g L^{-1} as $CaCO_3$. A methanogenic inhibitor (2-bromoethanesulphonic acid sodium salt, BES, 20 mmol L^{-1}) was also added. Finally, distilled water was added to make up the volume to 230 mL.

Once the reactors were loaded, they were purged with nitrogen gas for about 1 minute to remove any residual oxygen, and then sealed. The reactors were placed at 37 ± 1 °C (mesophilic operation) in a thermostatic incubator (WTC™ Binder BD 115) for about 30 days. Mixing was provided by a magnetic stirring system (VELP Scientifica™ Microstirrer, speed rate of about 700 RPM), thus ensuring a completely mixed condition.

A first set of experiments was conducted to investigate the potential of VFA production in the batch fermentation of all selected wastes, and to evaluate which ones present a better ability to acidify under anaerobic conditions. On a second set, a factorial experiment was conducted for one of the organic streams that presented higher acidogenic potential (cheese whey) in order to evaluate the effect of two selected factors – initial alkalinity and food-to-microorganism ratio (F/M) – on the VFA production and composition profile. In this latter set of experiments, 16 batch assays (3 replicates for each one) were conducted at the same previous tested conditions (mesophilic temperature, and biomass concentration of 2 g L^{-1} as VSS). Added alkalinity varied between 1 and 7 g L^{-1} as $CaCO_3$, and F/M ratio varied between 2 and 10 g COD g^{-1} VSS, thus comprising a full factorial experiment at 4 levels for each of the factors. Second order models were fitted to the experimental results.

Analytical methods

All the analyses were performed in accordance with standard analytical procedures.[16] Alkalinity and pH were measured with an automatic titrator Mitsubishi™ GT, according to methods 2320-B and 4500-H^+B.[16] For liquid samples Chemical Oxygen Demand (COD) was measured by a colorimetric method using a spectrophotometer equipment Aqualytic™ COD Vario PC compact (method 5220-D[16]). For solid samples, COD was determined by an open reflux method (5220-B[16]). The solids analyses were performed with glass microfibre filters (Reeve Angel™ grade 403), analytical balance Precisa™ XB120, drying oven WTC™ Binder E28 and muffle furnace Termolab™ Fuji PXR-9 (methods 2540-B, 2540-D and 2540-E[16]).

VFA were analysed by gas chromatography through the injection of 0.5 μL of filtered sample containing 10 % (v/v) of formic acid (Panreac™) in a gas chromatograph Chrompack™ CP9001, with injector set to 300 °C, flame ionisation detector set to 240 °C, and a 25 m x 0.25 mm Chrompack™ CPSIL-5CB column. The analyses were performed at constant flow, where helium (8 mL min^{-1}) and nitrogen (30 mL min^{-1}) were used as carrier and make-up gases, respectively. The temperature program used was: 1 minute at 70 °C, rise of 20 °C min^{-1} to 100 °C and then kept for 2 minutes; rise of 10 °C min^{-1} to 140 °C, and kept for 3 minutes (11.70 minutes total running time). Calibration curves were obtained by injecting standards of acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric, and n-caproic acids (Riedel-de Haën™).

Calculations

The degree of acidification (DA) was the main parameter used to evaluate the acidogenic potential of each organic waste stream. It was calculated through the quotient between the sum of each individual VFA produced expressed as COD equivalents at the maximum point (tVFA) and the influent total COD. Since some of the organic waste streams already presented some VFA content, it was then considered a net DA that is referred to the amount of VFA produced. It was calculated by the same quotient but considering the maximum tVFA concentration achieved after fermentation minus the initial tVFA present in the organic current. Regarding the potential valorisation into PHA, where the VFA composition plays an important role, an additional parameter (odd-to-even VFA ratio) was considered for the evaluation and it was defined as the sum of odd-equivalent carboxylic acids formed (propionic and n-valeric acids) divided by the sum of even-equivalent carboxylic acids formed (acetic, iso-butyric, n-butyric, iso-valeric, and n-caproic acids).

Response surface methodology was applied to find predictive scenarios for optimal operation and potential scale-up of the acidogenic process. Results for either the degree of acidification or the odd-to-even VFA ratio achieved in the second experimental set were modelled according to Eq. 1:

$$E(z)=\beta_0+\beta_1x_1+\beta_2x_2+\beta_{1,2}x_1x_2+\beta_{1,1}x_1^2+\beta_{2,2}x_2^2 \quad (1)$$

where $E(z)$ is the response variable, x_1 is the F/M ratio (in g COD g^{-1} VSS), x_2 is the alkalinity added (in g L^{-1} as $CaCO_3$), β_0 is the model constant, β_1 and β_2 are linear coefficients (main effects), $\beta_{1,2}$ is a cross-product coefficient (interaction) and $\beta_{1,1}$ and $\beta_{2,2}$ are quadratic coefficients.[17] Regressions were performed using the software StatSoft Statistica™.

Results and discussion

Acidogenic potential of the organic waste streams

A first set of experiments was performed to evaluate the acidogenic potential of eight organic streams: cheese whey; sugarcane molasses; organic fraction of municipal solid wastes (OFMSW); glycerol and soapy slurry residues from biodiesel production; winery wastewater; olive mill effluent (OME); and landfill leachate. Fig. 1a presents the total volatile organic acid (tVFA) concentrations measured throughout the tests, and Fig. 2a shows the maximum net degree of acidification achieved in each experiment. It can be seen that the maximum tVFA concentration achieved and the time needed to reach this maximum varied among the different waste streams studied. After these maximums were achieved, VFA concentrations remained constant or decreased slightly throughout the experiments. In acidogenic reactors, substrates were expected to be converted into intermediates (VFA and alcohols), which account for the soluble COD in the liquid phase.[3]

During all tests, fermentation started immediately after inoculation. VFA concentration increased rapidly up to 7 days for almost all the experiments. In the assay with winery effluent, the highest rising on VFA production was only observed at up to 13 running days. The increase of non-VFA components in the anaerobically treated winery effluent was previously described by Ganesh *et al.*[18] that reported an almost constant level of VFA (2 – 3 g L^{-1} as COD) regardless of the increasing organic loading rate above 15 g $L^{-1}d^{-1}$ (as COD), and observed the build-up of other anaerobic intermediates or non-acidified organic matter at such high loading conditions. However, the production of VFA from the winery effluent in this study increased almost 4-fold between days 7 and 14, up to 1070 mg L^{-1} as COD. For glycerol waste, a very similar effect was

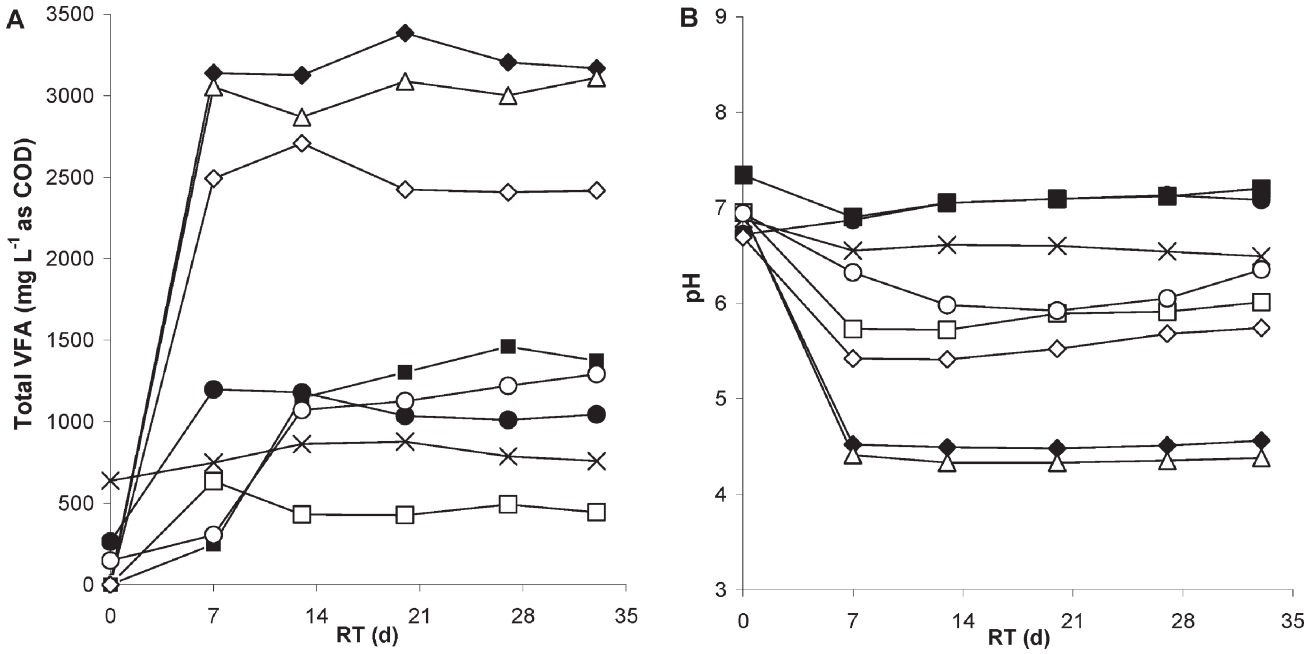

Fig. 1 – *(a) Total VFA concentration vs. time during the acidification assays; (b) pH profiles vs. time during the acidification assays: (◆) cheese whey; (△) sugarcane molasses; (◇) OFMSW; (■) glycerol waste; (○) winery effluent; (●) OME; (×) landfill leachate; (□) soapy waste*

observed, although in this case it was most likely due to a lag time needed to acclimatise the biomass. Moreover, tVFA produced from both substrates slightly increased until the end of the experiments.

Cheese whey, sugarcane molasses and OFMSW presented the highest values for the maximum tVFA produced, in the range 2400 – 3400 mg L^{-1} as COD, whereas all the other organic substrates resulted in much lower values, in the range 450 – 1400 mg L^{-1} as COD. The higher VFA productions for the aforementioned substrates are in agreement with the observed trend for pH values (Fig. 1b). After day 7, sugarcane molasses, cheese whey and OFMSW presented the lowest pH values among the substrates tested, around 4.35, 4.50 and 5.60 respectively. In general, pH dropped to values below 6.0 throughout the assays, with the exception of glycerol waste and olive mill effluent, which remained near 7.0 during the entire experiment. Table 2 presents mass carbon balances, where the fraction tVFA/sCOD represents the readily fermentable organic fraction present in the final fermentate. The net degree of acidification (Fig. 2a) for

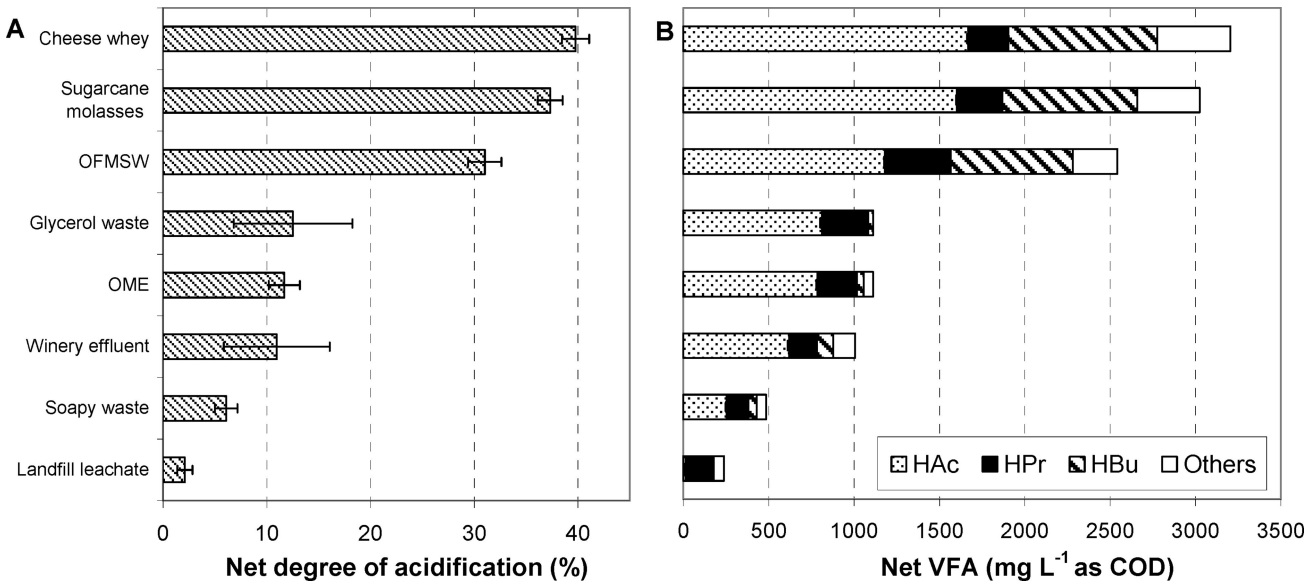

Fig. 2 – *Net acidification yields: (a) average acidification yield on substrate fed; (b) average composition of VFA produced; HAc – acetic acid; HPr – propionic acid; HBu – butyric acid*

Table 2 – *Mass balance for the acidogenic fermentation experiments (mean ± standard deviation)*

	Initial COD (mg L^{-1})	Maximum tVFA (mg L^{-1} as COD)	DA (%)	tVFA/sCOD (%)
Cheese whey	8054 ± 105	3374 ± 138	39.8 ± 1.3	51.6 ± 3.8
Sugarcane molasses	8101 ± 60	3110 ± 124	37.3 ± 1.2	42.1 ± 2.8
OFMSW	8142 ± 126	2707 ± 82	31.0 ± 1.6	32.4 ± 3.0
Wasted glycerol	8125 ± 138	1460 ± 196	12.5 ± 5.7	18.2 ± 1.9
Olive mill effluent	8128 ± 87	934 ± 113	11.7 ± 1.5	13.6 ± 2.4
Winery effluent	8009 ± 46	1144 ± 175	11.0 ± 5.1	16.2 ± 2.3
Soapy slurry waste	7969 ± 115	634 ± 98	6.1 ± 1.1	8.8 ± 1.7
Landfill leachate	8025 ± 98	240 ± 95	2.1 ± 0.7	3.3 ± 1.5

glycerol waste and olive mill effluent was quite low (in the range 12 – 13 %), and the fraction of soluble COD present as VFA after fermentation was also low (13.6 – 18.2 % in Table 2), indicating a very low readily fermentable organic fraction.

Concerning cheese whey, sugarcane molasses and OFMSW, a significant fraction of the sCOD was present as VFA after fermentation (51.6 %, 42.1 % and 32.4 % respectively), indicating a high readily fermentable potential of these three substrates. For glycerol waste, olive mill effluent and winery effluent, the fraction of VFA present in the final sCOD was much lower (between 13.6 and 18.2 %), whereas for soapy slurry and leachate only less than 10 % of the final sCOD could be recovered as VFA. All these behaviours were in agreement with the net degrees of acidification (Fig. 2a), where three distinct patterns can be distinguished: high acidification potential substrates (cheese whey, sugarcane molasses and OFMSW, presenting degrees of acidification between 31 and 40 %), medium acidification potential substrates (glycerol waste, OME and winery effluent, with degrees of acidification between 11 and 13 %) and low acidification potential substrates (soapy waste and landfill leachate, presenting degrees of acidification between 2 and 6 %).

Composition of the fermentation products

Regarding the composition of the VFA produced (Fig. 2b) the predominant fermentation products were acetic, propionic and n-butyric acids for all the organic waste streams with the exception of landfill leachate. In some cases, small amounts of n-valeric, iso-valeric or n-caproic acids were also observed. Iso-butyric acid was only produced in the experiment with landfill leachate. For the three most readily fermentable organic currents, acetic acid (46 – 53 % of tVFA) and n-butyric acid (26 – 28 % of tVFA) were the predominant VFA. These were also the only substrates where a significant build-up of VFA with longer carboxylic chains was observed (10 – 14 %), mainly consisting of n-caproic and iso-valeric acids.

The build-up of longer chain VFA was in agreement with the low pH values observed in the experiments with these three substrates (Fig. 1b), which was also observed in other studies. For the acidogenic fermentation of molasses, Albuquerque *et al.*[19] reported that acetic and propionic acid concentrations were lower when pH value decreased from 7 to 5, while butyric and valeric acid concentrations significantly increased. For cheese whey fermentation, the predominance of acetic and butyric acids was also reported in the same extent by Davila-Vasquez *et al.*,[20] although the authors have observed a relative increase of pH value (around 6). Those authors attributed that observation to the ammonia produced during the anaerobic digestion of cheese whey. For the digestion of OFMSW, Capela *et al.*[21] observed a high tendency for VFA build-up, with the predominance of acetic, n-butyric and propionic acids, corresponding to the lowest pH value observed (6.7). In fact, lower operating pH favours the production of longer chain fatty acids, since under these more acidic conditions there are more reducing equivalents available to be incorporated into the fatty acid chains.[22]

The significant presence of n-butyric acid (or even heavier VFA) was not detected for the medium and low acidogenic potential substrates, as can be observed from Fig. 2b. For glycerol waste and OME, VFA compositions were very similar, with fractions of acetic and propionic acids of 71 – 73 % and 21 – 24 % respectively. Although resulting in similar net productions of VFA, glycerol and OME presented slightly different behaviours along the retention time (Fig. 1a). OME rapidly started to produce organic acids up to a maximum of 1200 mg L^{-1} (as COD) at day 14 which after that decreased grad-

ually to 1050 mg L^{-1} (as COD) at the end of the experiment. In turn, glycerol experiment needed more time for adaptation of biomass, but the VFA production increased until almost the end of the experiment (maximum value of 1460 mg L^{-1} as COD at day 28). The DA of 13 % achieved for glycerol in this study was lower than 27 – 29 % reported by Forrest *et al.*,[23] who observed the predominance of acetic and butyric acids, but the absence of propionic acid. Different yields and compositions possibly resulted because the thermophilic batch fermentation carried out by those authors could shift the VFA profiles of the same substrate compared to the mesophilic digestion performed in the present study. For the batch acidification of OME, Dionisi *et al.*[24] reported higher concentrations of VFA, but using a much higher initial substrate concentration (up to 70 g L^{-1} as COD), which resulted in higher degrees of acidification (22 – 44 % versus 12 % in this study). In their study, acetic and butyric acids were the predominant forms of acidity, while propionic acid was measured to a lesser extent (7 – 14 %).

For the soapy slurry waste, maximum tVFA concentration of 634 mg L^{-1} (as COD) was observed almost immediately after the start-up of the fermentation process (day 7), and it remained almost constant until the end of the experiment (Fig. 1a). Fermentation of soapy waste resulted in a degree of acidification of 6.1 %, with pH values between 5.75 and 6. The main VFA products were acetic (52 %) and propionic (26 %) acids. For this current, the degree of acidification may have been strongly affected by the presence of inhibitory amounts of sodium cation from the alkaline catalyst used for saponification (NaOH), which is toxic for the anaerobic ecosystem, or even the presence of other toxic molecules generated during saponification.[25]

Landfill leachate was the organic waste that resulted in a minimal VFA production among all fermented substrates yielding an almost insignificant net degree of acidification (2.1 %). Landfill leachates typically present very low BOD_5/COD ratios (0.09 – 0.12), which indicate that their organic content is difficult to biodegrade. In addition, leachates have been mentioned as toxic to microorganisms and therefore physicochemical treatments are usually preferred rather than biological processes.[26] However, anaerobic treatment of leachate is still possible if biomass is conveniently acclimatised and an adequate organic load of leachate is provided to the biological reactor.[27] Considering the net production of VFA from landfill leachate in the present study, only 216 mg L^{-1} (as COD) of volatile acidity were generated, mainly propionic (77 %) and small amounts of iso-butyric and n-valeric acids. At the beginning of the fermentation process, leachate already presented about 600 mg L^{-1} (as COD) of acetic acid (6 % of sCOD). The acetic acid content remained almost constant during the fermentation process. In addition, pH did not decrease significantly (average value of 6.6), highlighting its poor ability to be fermented into volatile acids without a convenient biomass acclimatisation.

Effect of alkalinity and F/M ratio on the acidogenic fermentation of cheese whey

A first assessment on the acidogenic potential of the organic waste streams under study permitted to infer which ones presented a better valorisation potential in terms of VFA production. Considering the high degree of acidification achieved for cheese whey fermentation, this organic substrate was selected for closer examination and to obtain additional information concerning the optimisation of the environmental conditions that maximise VFA production. It has been demonstrated that the amount and composition of VFA produced under acidogenic fermentation can be affected by environmental conditions such as pH, organic loading rate, retention time, or temperature.[28]

Composition of the VFA achieved in the acidogenic fermentation is of major concern regarding their effective valorisation into PHA biosynthesis, for instance. Given that the production of organic waste streams is often sensitive to seasonal variations, and regarding their potential valorisation into PHA, where the composition of the VFA fed as substrate is a crucial parameter, acidification processes must be accomplished through the manipulation of the operational parameters, in order to achieve a coherent and appropriate VFA profile.[19]

A full factorial experiment was conducted with cheese whey to evaluate the influence of initial alkalinity (1, 2, 5, and 7 g L^{-1} as $CaCO_3$) and F/M ratio (2, 4, 7, and 10 gCOD g^{-1}VSS) upon VFA production and profile. Response surface models were applied to the experimental results of both the degree of acidification (DA) and odd-to-even VFA ratio. Fig. 3 shows the contours of the quadratic models fitted to the experimental data and Table 3 presents the regression coefficients as well as the parameters for evaluating the quality of the fitting. The correlation coefficients achieved for data fitting of the degree of acidification and the odd-to-even VFA ratio were 0.735 and 0.657 respectively. In the test for the significance of the regression (ANOVA), the rejection of the null hypothesis implies that at least one of the independent variables contributes significantly to the model. For both regressions, this test resulted in p-values below 0.05, meaning that experimental behaviours were well described by the computed models at that significance level. Since the experiments were conducted in triplicate, a lack-of-fit test was performed to examine if the

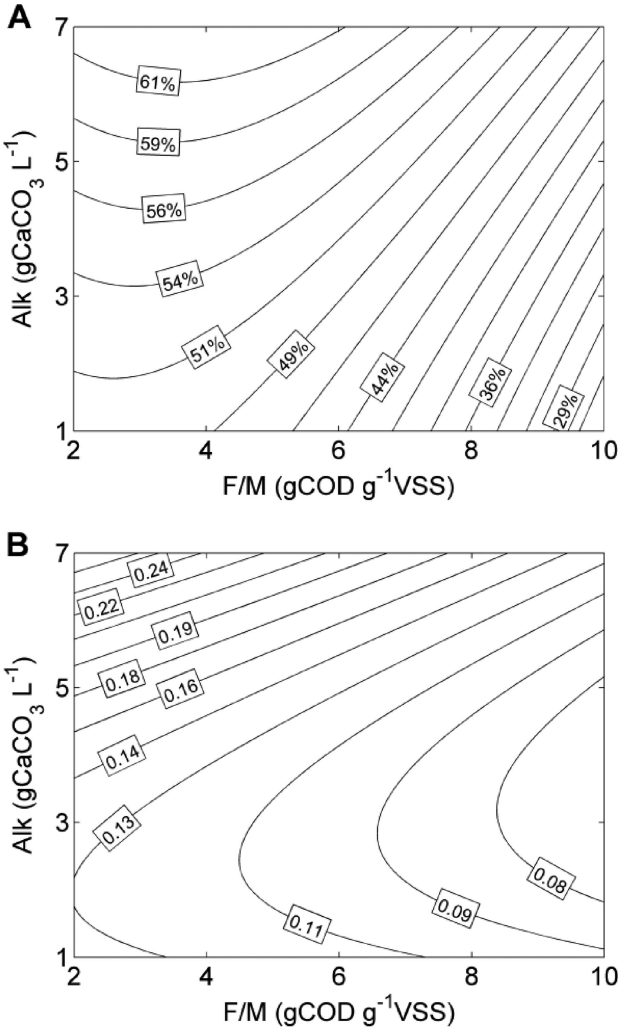

Fig. 3 – *Response surface contours calculated from the factorial batch fermentation of cheese whey: (a) degree of acidification (%); (b) odd-to-even ratio of carboxylic chains produced*

model was estimated with sufficient precision to yield satisfactory predictions (null hypothesis). The p-value obtained in this test for DA regression was higher enough to consider that the tentative model adequately described the data. However, for the odd-to-even ratio the p-value obtained for the lack-of-fit test was very small, which may not ensure high precision of the calculated model despite its verified significance.

Table 3 – *Fitting coefficients and evaluation of regression*

Regression coefficients	DA	Odd-to-even VFA ratio
β_0	0.577	0.118
β_1	-3.32×10^{-3}	2.23×10^{-3}
β_2	-1.96×10^{-2}	-1.07×10^{-2}
$\beta_{1,2}$	3.08×10^{-3}	-2.96×10^{-3}
$\beta_{1,1}$	-3.28×10^{-3}	-9.19×10^{-5}
$\beta_{2,2}$	4.22×10^{-3}	6.56×10^{-3}
Adjusted r^2	0.735	0.657
ANOVA (p-value)	1.75×10^{-4}	1.92×10^{-3}
Lack-of-fit (p-value)	0.4251	2.01×10^{-4}

Regarding the degree of acidification (Fig. 3a), the maximum predicted values (around 61 %) were observed for the highest initial alkalinity (7 g L^{-1} as $CaCO_3$) combined with a moderate substrate concentration (F/M = 4 gCOD g^{-1}VSS). As it can also be seen, alkalinity presented lower influence than F/M ratio, since increasing alkalinity from 1 to 7 g L^{-1} (as $CaCO_3$) led only to an increase in the degree of acidification from 50 to 63 % when the F/M was 2 gCOD g^{-1}VSS. Conversely, the contour along the F/M axis was much more pronounced, indicating that under low alkalinity supplies, an increase in the F/M ratio from 2 to 10 gCOD g^{-1}VSS resulted in a sharp decrease of the degree of acidification (from 50 to less than 29 %). Therefore, the F/M ratio can be considered to induce an antagonistic effect (confirmed by the negative values of β_1 and $\beta_{1,1}$ in Table 3), mainly when combined with low alkalinity, since the latter was quickly consumed due to a rapid conversion of the organic components of whey into VFA. In turn, alkalinity presented a synergistic effect on the DA (positive value of $\beta_{2,2}$ in Table 3), mainly when combined with increasing F/M ratios (positive value of the interaction coefficient $\beta_{1,2}$ in Table 3). At the highest F/M ratio, an increase in alkalinity from 1 to 7 g L^{-1} as $CaCO_3$ resulted in an increase in DA from 26 % to around 45 %.

From Fig. 3b, a wide variation can be observed on the odd-to-even VFA ratio (0.08 – 0.26) within the batch experiments, which probably led to a lower correlation coefficient in the computed model. Generally, the initial alkalinity presented more influence on the odd-to-even ratio than the F/M ratio, which was reflected by the higher magnitude of the coefficients β_2 and $\beta_{2,2}$ in Table 3, when compared to β_1 and $\beta_{1,1}$. In all the experiments, acetic and n-butyric acids were always predominant (accounting between 70 and 90 % of tVFA), but when low F/M ratios were combined with high alkalinity, significant amounts of propionic and n-valeric acids were also produced (up to 500 mg L^{-1} as COD), which resulted in an increased odd-to-even ratio (up to 0.26). In turn, the lowest odd-to-even ratios were observed for higher F/M ratios combined with a moderate alkalinity addition (3 g L^{-1} as $CaCO_3$), mainly due to the build-up of butyric acid, which was previously observed in the first set of experiments (Fig. 2b) and in other results reported.[20, 28–30] Butyric acid can be more toxic than acetic acid in a hydrogen-saturated system, and it is known that inhibition can be mitigated by keeping the pH values above 4.8 to help decrease the concentration of the undissociated form of butyric acid.[20] This inhibition might be the reason

why lower degrees of acidification were observed for high F/M ratios combined with low alkalinity addition (Fig. 3a), as the final pH value was very low in those experiments.

Cheese whey is widely available and its world production is estimated at around 85 million tons per year, although only 60 % is currently being valorised in food applications.[31] Cheese whey surplus is often discharged in wastewater treatment plants, where its high organic content can result in process unbalances in addition to the carbon waste. Nowadays, the polyhydroxyalkanoates market is being driven by the search for cost-effective approaches, such as the use of microbial mixed cultures combined with the use of inexpensive fermented substrates, aiming to significantly decrease the cost of these biopolymers compared to their production through pure cultures using single substrates.[32] Considering the increasing demand for fermented substrates suitable for polyhydroxyalkanoates production in open mixed cultures that are able to deal with a wide variety of carbon sources, cheese whey has proven to be an interesting substrate for acidogenic fermentation since different VFA can be generated with considerable process yields (DA up to 63 %). Towards a more sustainable technology, acidogenic fermentation is now faced as a platform that consolidates the biorefinery concept rather than a treatment stage.

Conclusions

Batch acidification of eight organic streams resulted in different VFA productions and profiles. The studied substrates were ranked into three categories according to the degree of acidification (DA) achieved: high acidogenic potential with DA up to 40 % (cheese whey, molasses and OFMSW), medium acidogenic potential with DA up to 13 % (glycerol, OME and winery effluent), and low acidogenic potential with DA up to 6 % (soapy waste and leachate). For the former waste streams, the build-up of heavier VFA such as butyric acid significantly occurred. In addition, DA for cheese whey can be maximised up to 63 % under high alkalinity concentrations (5 – 7 g L^{-1} as $CaCO_3$) and moderate to low substrate concentrations (F/M of 3 – 4 gCOD g^{-1}VSS).

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List of abbreviations and symbols

3HV – 3-hydroxyvalerate
AD – Anaerobic digestion
Alk – Alkalinity (g L^{-1} as $CaCO_3$)
BES – 2-Bromoethanesulfonic acid sodium salt
COD – Chemical oxygen demand (mg L^{-1})
COD_{fed} – Chemical oxygen demand fed (mg L^{-1})
DA – Degree of acidification (%)
F/M – Food-to-microorganism ratio (gCOD g^{-1}VSS)
HAc – Acetic acid
HBu – Butyric acid
HPr – Propionic acid
OFMSW – Organic fraction of municipal solid wastes
OME – Olive mill effluent
PHA – Polyhydroxyalkanoates
PHB – Poly-3-hydroxybutyrate
RT – Retention time (d)
sCOD – Soluble Chemical Oxygen Demand (mg L^{-1})
tCOD – Total Chemical Oxygen Demand (mg L^{-1})
tVFA – Total volatile fatty acids (mg L^{-1})
VFA – Volatile fatty acids (mgL^{-1} as COD)
VSS – Volatile suspended solids (g L^{-1})

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